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Sample extraction and metabolic profiling

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An abbreviated version of this protocol was published in eLIFE in Oct 2019

Metabolic stress is a primary pathogenic event in transgenic *Caenorhabditis elegans* expressing pan-neuronal human amyloid beta

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Detailed protocol

C. elegans cultivation

1. Grow 1500-2000 worms on a 145mm petri dish. Make sure the worms have enough food until the day of harvest. (note: this amount of worm is sufficient for one sample. Prepare more plates of worms to get biological repeats)
2. On the day of harvest, wash worms off the NGM plate using M9 buffer, followed by two additional washes to remove excess bacteria.
3. Transfer the worm pellet to the homogenizing tube (or a 1.5ml Eppendorf tube) and store the pellet in -80C until homogenisation.

Homogenisation of *C. elegans*

1. Add 50 % acetonitrile, 0.3 % formic acid to the C. elegans pellet. A good starting volume is 1ml, reduce or concentrate later if mass spectrometer sensitivity is low.
2. Homogenise using recommended programme prescribed by bead mill equipment, at 4^{o}C.
3. Keep 30ul of sample homogenate for protein quantification and normalize the metabolite results by protein concentration.

Amino acid LC-MS assay

1. Take 50 µl of homogenate, spike with your preference of stable isotopes of amino acids and acylcarnitines, vortex.
2. Add 400ul of methanol for extraction of metabolites, vortex, centrifuge max speed at 4^{o}C for 10 min.
3. Take 10 µl of the supernatant, dry using nitrogen gas, and add 100 µl of butanol-HCl (3M) (Sigma Aldrich).
4. Incubate mixture at 65 ^{o}C for 15 min
5. Dilute sample 50 times in water and run directly on LC-MS by injecting 4 µl of the sample.
6. Amino acids were separated using a C18 column (Phenomenex, 100 x 2.1 mm, 1.6 µm, Luna Omega) on a Agilent 1290 Infinity LC system (Agilent Technologies, CA, USA) coupled with quadrupole-ion trap mass spectrometer (QTRAP 5500, AB Sciex, DC, USA). Mobile phase A (Water) and Mobile phase B (Acetonitrile) both containing 0.1% formic acid were used for chromatography separation. The LC run was performed at a flow rate of 0.4 ml min^{-1} with initial gradient of 2% B for 0.8 min, then increased to 15% B in 0.1 min, 20% B in 5.7 min, 50% B in 0.5 min, 70% B in 0.5 min, followed by re-equilibration of the column to the initial run condition (2% B) for 0.9 min. All compounds were ionized in positive mode using electrospray ionization. The chromatograms were integrated using MultiQuant™ 3.0.3 software (AB Sciex, DC, USA).

Acyl carnitine LC-MS assay

1. From step 2 of the amino acid assay, take 100 µl of supernatant, dry using nitrogen gas, and add 100 µl of methanol-HCl (3M) (Sigma Aldrich).
2. Incubate mixture at 50 ^{o}C for 30min.
3. Dry again using nitrogen gas, and reconstitute sample in 200 µl of 80% methanol.
4. Inject 4 µl of the sample and run directly on LC-MS by flow analysis (without column), using 80 % methanol and 0.1% formic acid as mobile phase with a flow rate of 0.4ml/min.

Organic acid GC-MS assay

1. Centrifuge 300 µl of homogenate and retrieve supernatant.
2. Add 10 µl of stable isotopes of organic acids as internal standards and 10 µl of O-Ethylhydroxylamine HCL to samples. Incubate for 30mins at room temperature.
3. Add 50ul of 6M HCL and saturate samples with NaCl. Vortex and centrifuge.
4. Add 500 µl of ethyl acetate. Extract the top layer. Dry using nitrogen gas and add 5ul of pyridine and 50uL of BSTFA.
5. Incubate mixture at 85 ^{o}C for 30min.
6. Run sample on a GC single quad mass spectrometry. (Agilent 5975C mass spec and 6890 GC machine.)
7. Trimethylsilyl derivatives of organic acids were separated by gas chromatography on an Agilent Technologies HP 7890A and quantified by selected ion monitoring on a 5975C mass spectrometer using stable isotope dilution. The initial GC oven temperature was set at 70 ^{o}C, and ramped to 300 ^{o}C at a rate of 40 ^{o}C/min, and held for 2 min.
8. Analyze result on a mass hunter program.

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Gruber, J. (2020). Sample extraction and metabolic profiling. Bio-protocol Preprint. bio-protocol.org/prep534.
2. Teo, E., Ravi, S., Barardo, D., Kim, H., Fong, S., Cazenave-Gassiot, A., Tan, T. Y., Ching, J., Kovalik, J., Wenk, M. R., Gunawan, R., Moore, P. K., Halliwell, B., Tolwinski, N. and Gruber, J.(2019). Metabolic stress is a primary pathogenic event in transgenic *Caenorhabditis elegans* expressing pan-neuronal human amyloid beta. eLIFE. DOI: 10.7554/eLife.50069